

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Cantor *et al.*

Serial No.: 09/880,988

Confirmation No.: 5954

Filed: : June 13, 2001

For: *USE OF NUCLEOTIDE ANALOGS IN
THE ANALYSIS OF OLIGONUCLEOTIDE
MIXTURES AND IN HIGHLY
MULTIPLEXED NUCLEIC ACID
SEQUENCING*

Art Unit: 1634

Examiner: Chakrabarti, Arun K.



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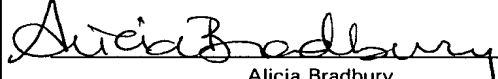
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Alicia Bradbury

ATTACHMENT TO THE AMENDMENT

1. Marked up copy of amended claims per 37 C.F.R. §1.121.

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MARKED UP CLAIMS (37 C.F.R. § 1.121)

Please amend claims Claims 4, 6, 17, 27, 28, 31, 33, 36 and 42 as follows:

4. (Amended) The method of claim 1, wherein a mass-matched deoxynucleotide is deoxyinosine, 5-nitroindole, 3-nitropyrrole, 3-methyl 7-propynyl isocarbostyryl, 5-methyl [isocarbostyryl] isocarbostyryl or 3-methyl [isocarbostyryl] isocarbostyryl.

6. (Amended) The method of claim 5 that is a method for determining nucleotide sequences of a plurality of target nucleic [acids] acid molecules, comprising:

synthesizing extension products of the target nucleic acid in the presence of chain terminating nucleotides and mass-matched nucleotides;

determining the mass of each extension product; and

calculating a mass shift from a period for the mass of each extension product,

whereby the nucleotide sequences of the target nucleic acids are

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AMENDED CLAIMS

determined by determining the nucleotide that corresponds to each mass shift.

17. (Amended twice) A method for detecting [a] one or a plurality of target nucleic acid(s) molecules or one or a plurality of nucleotides therein [molecules], comprising:

(a) copying the target nucleic acid molecule(s) in the presence of a pair-matched set of nucleotides;

(b) denaturing the resulting copies of the target(s) to produce single-stranded templates;

(c) annealing and ligating one or a plurality of partially duplex hairpin primers to the single-stranded template(s);

(d) extending the primer(s) in the presence of chain terminating nucleotides and pair-matched nucleotides to produce extension products, wherein the extension products follow a periodic mass distribution that is determined by the mass of the pair-matched nucleotide set; and

(e) detecting each of the targets or nucleotides therein by virtue of the mass shift of each extension product from its corresponding periodic reference mass.

27. (Amended) A method for detecting a plurality of target nucleic acid molecules in a sample containing nucleic acid molecules, comprising:

preparing a composition containing a plurality of pair-matched nucleic acid molecules or mass-matched nucleic acid molecules from a sample comprising the target nucleic acid molecules;

analyzing the resulting composition by mass spectrometry; and
detecting target nucleic acid molecules.

28. (Amended) A process for detecting a mutation in a target nucleic acid sequence in a target nucleic acid molecule, in a sample, comprising:

a) hybridizing [a nucleic acid molecule] a primer to nucleic acid molecules in the sample, thereby producing a hybridized primer and a molecule from the sample, wherein:

the nucleic molecules from the sample are optionally immobilized[;] and the primer is complementary to a sequence in the target nucleic acid sequence that is adjacent to the region suspected of containing a mutation sequence;

b) contacting the hybridized primer with a composition comprising mass-matched deoxyribonucleoside triphosphates and a chain terminating nucleotide selected from a dideoxyribonucleoside triphosphate or a 3'-deoxynucleoside triphosphate and optionally one or more deoxyribonucleoside triphosphates, such that the hybridized primer is extended until a chain terminating nucleotide is incorporated, thereby producing an extended primer; and

c) determining the mass of the extended primer, thereby determining whether a mutation is present in the target nucleic acid sequence.

31. (Amended) A process for detecting mutations in a plurality of target nucleic acid sequences in a sample, comprising:

a) hybridizing a plurality of primers to nucleic acid molecules in the sample, thereby producing [a] hybridized primers, wherein:

the nucleic acid molecules from the sample are optionally immobilized[;] and each primer is complementary to a sequence of a target nucleic acid sequence that is adjacent to a region suspected of containing a mutation sequence;

b) contacting the hybridized primers with a composition comprising a chain terminating nucleotide selected from a mass-matched dideoxyribonucleoside triphosphate or a 3'-deoxynucleoside triphosphate and one or more deoxyribonucleoside triphosphates, such that the hybridized primers are extended until a chain terminating nucleotide is incorporated, thereby producing an extended primer; and

c) determining the mass of the extended primers, thereby

determining whether mutations are present in the target nucleic acid sequences.

33. (Amended) The method of claim 31, wherein the [mass] masses of the extended primers are determined by mass spectrometry.

36. (Amended twice) A method for detecting a plurality of target nucleic acid sequences, comprising the steps of:

a) hybridizing a primer or plurality thereof to nucleic acid molecules comprising target nucleic acid sequences, wherein the primers can be extended in a 3' direction towards the target nucleic acid sequence, and wherein the 5' end of the hybridized mass-matched nucleic acid molecules can be selectively cleaved from the extension product;

b) extending the primers in the presence of mass matched deoxyribonucleotides and a polymerase to produce extension products;

c) selectively cleaving the 5' end of the primers from the extension products to produce portions of the primers and cleaved extension products; and

d) detecting the cleaved extension products.

42. (Amended) A method for detecting a target nucleic acid sequence, comprising:

a) hybridizing first and second primers to a nucleic acid molecule containing the target nucleic acid sequence, wherein a primer contains a selectively cleavable site at its 3' end;

b) extending the primers in the presence of mass-matched nucleotides;

c) cleaving the resulting product at the selectively cleavable sites; and

d) analyzing the masses of the cleavage products, whereby the target sequence is detected.